Eur Respir J 2008; 31: 1213–1220
DOI: 10.1183/09031936.00082407

Ciclesonide improves measures of small airway involvement in asthma

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ABSTRACT: Ciclesonide is delivered as a small-particle inhaled corticosteroid and improves lung function and airway hyperresponsiveness. The objective of the present study was to assess whether ciclesonide can specifically improve small airway function in asthma.

A total of 16 mild-to-moderate asthma patients (seven males; median (range) age 39 (19–56) yrs and forced expiratory volume in one second (FEV_1) 89 (62–120)% predicted) were randomised to 5 weeks' treatment with placebo or 320 μg ciclesonide once daily. The following small airway parameters were assessed: mean forced expiratory flow between 25 and 75% of forced vital capacity (FVC), percentage fall in FVC at provocative dose of adenosine-5′-monophosphate and of methacholine (MCh) causing a 20% fall in FEV_1, expiratory lung volume on computed tomography (CT) scan after MCh challenge, single-breath nitrogen closing volume and alveolar exhaled nitric oxide (eNO).

Seven subjects received placebo and nine received ciclesonide. Both alveolar eNO and CT measurements of expiratory lung volume after MCh challenge decreased significantly with ciclesonide (median (range) decrease 4.4 (54.8–1.4) ppb and 59 (1,569– -117) mL, respectively), and compared with placebo (-0.4 (7.3– -3.4) ppb and -121 (20– -236) mL respectively). Ciclesonide did not significantly improve other small airways parameters.

Inflammation and patency of small airways, reflected by alveolar exhaled nitric oxide and air trapping on computed tomography scan, both improve with ciclesonide even in this small number of patients. This indicates that ciclesonide exerts anti-inflammatory effects on small airways.

KEYWORDS: Asthma, ciclesonide, small airways

Asthma is a chronic inflammatory disease of the airways and anti-inflammatory treatment with inhaled corticosteroids (ICS) constitutes the cornerstone of asthma management. Nevertheless, a considerable subset of asthma patients does not benefit from ICS or gain optimal asthma control [1–3]. It can be speculated that inflammation of the small airways contributes to the poor asthma control observed, since small airways are not directly reached by conventional ICS [4].

The small airways, *i.e.* airways with an internal diameter <2 mm, have not always been considered important in asthma. After having been dubbed 'the quiet zone' by MEAD *et al.* [5] because they contributed merely 10% to total airway resistance, the small airways have regained attention over the past 15 yrs due to their role in asthma. At present, it is acknowledged that increasing physiological and pathological evidence exists that inflammation of the small airways is similarly and often even more pronounced than in larger airways in severe asthma [6, 7]. This new insight in the importance of small airway inflammation in asthma has led to the introduction of ICS with small-particle formulations that target this site of inflammation. Ciclesonide (Alvesco®; Nycomed BV, Hoofddorp, the Netherlands) is such an ICS, as it is formulated as a solution delivered *via* a hydrofluoroalkane-134a metered-dose inhaler. A labelling study showed that a high fraction of ciclesonide (52%) is deposited in the lung. Additionally, three-dimensional single photon emission computed tomography (3D SPECT) analysis revealed that the highest ciclesonide deposition was found in peripheral regions of the lung, *i.e.* the zones with small airways and alveoli [8].

Ciclesonide has been demonstrated to maintain asthma control [9] and improve lung function

For editorial comments see page 1145.
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Received:
July 04 2007
Accepted after revision:
February 04 2008

CLINICAL TRIAL
The present study is registered at clinicaltrials.gov (identifier NCT00163345).

STATEMENT OF INTEREST
Statements of interest for D.S. Postma and the study itself can be found at www.erj.ersjournals.co.uk/misc/statements.shtml

European Respiratory Journal
Print ISSN 0903-1936
Online ISSN 1399-3003

(forced expiratory volume in one second (FEV_1), peak expiratory flow and forced vital capacity (FVC)) in both mild-to-moderate and moderate-to-severe asthma [10]. Furthermore, ciclesonide reduces symptoms and airway hyperresponsiveness assessed with both methacholine (MCh) [11] and adenosine-5′-monophosphate (AMP) [12, 13].

Although it is known that ciclesonide improves lung function and inflammation [14–17], it is still unknown whether it specifically improves small airway function and inflammation. It has been demonstrated that ciclesonide reaches the small airways [8], therefore it can be hypothesised that ciclesonide improves small airway parameters in asthma. In order to determine the efficacy of ciclesonide, different parameters of small airway function and inflammation were evaluated in 16 mild-to-moderate asthma patients in a double-blind randomised, placebo-controlled pilot trial with 320 μg ciclesonide, once daily.

MATERIALS AND METHODS

Subjects

Subjects were recruited from the outpatient clinic of the Dept of Pulmonology (University Medical Center Groningen, Groningen, the Netherlands) and by advertisements in local papers. The local medical ethics committee reviewed and approved the study protocol, and the study was registered in a public trial database (clinicaltrials.gov identifier NCT00163345). All subjects gave their written informed consent.

Inclusion criteria

Subjects of either sex, between 18 and 60 yrs of age, with a history of asthma according to the Global Initiative for Asthma criteria [18] and using $<800\ \mu g \cdot day^{-1}$ of budesonide or equivalent were eligible for study participation. In addition, subjects were required to have: a baseline $FEV_1 \geqslant 60\%$ predicted [19]; bronchial responsiveness, defined as a provocative concentration causing a 20% fall in FEV_1 from baseline (PC_{20}), to both MCh and AMP of $\leqslant 4.9\ mg \cdot mL^{-1}$ and $\leqslant 40\ mg \cdot mL^{-1}$, respectively; and proven atopy defined by at least one positive skin prick test to 18 common aeroallergens.

Exclusion criteria

Current smokers or ex-smokers with cessation of smoking <1 yr prior to study participation or with >10 pack-yrs were excluded. In addition, subjects were not eligible if they had: 1) a history of chronic obstructive pulmonary disease (COPD) or other pulmonary or concomitant diseases expected to interfere with the study; 2) unstable asthma (defined as more than three exacerbations in the previous year or one exacerbation in the previous 2 months); 3) concomitant medication that was not allowed (*e.g.* oral corticosteroids within 4 weeks prior to study participation); 4) intolerance for short-acting β_2-agonists (SABA) or suspected hypersensitivity for ICS; or 5) females who were pregnant, lactating or lacking an effective method of contraception.

Study design

The present pilot study was designed as a double-blind, randomised, placebo-controlled, parallel-group trial (a schematic of the study is shown in figure 1). The study consisted of a 4-week pre-baseline period, for those pre-treated with ICS with or without a long-acting β_2-agonist (LABA), followed by a 2–3-week baseline period and a 5–6-week treatment period, depending on whether a bronchoscopy was performed. Treatment with ICS or LABA was withdrawn during the pre-baseline period and substituted with SABA only as rescue medication. Subjects who were treated with SABA only as rescue medication and who had an $FEV_1 \geqslant 60\%$ pred entered the study in the baseline period. After the 2–3-week baseline period, subjects demonstrating bronchial responsiveness to both MCh and AMP were randomised in order to receive either ciclesonide 320 μg once daily or placebo in the morning for 5–6 weeks. Randomisation was stratified for pre-treatment with or without ICS.

Small airways parameters

Alveolar exhaled nitric oxide fraction

Endogenous nitric oxide production is increased in asthma due to inflammation of airway epithelium [20]. Measurement of alveolar exhaled nitric oxide (eNO) reflects inflammation in small airways [21].

At two baseline visits, 1 week apart, and after treatment, eNO was measured at multiple flow rates ($30\ mL \cdot s^{-1}$, $50\ mL \cdot s^{-1}$, $100\ mL \cdot s^{-1}$ and $200\ mL \cdot s^{-1}$) using a NIOX (Aerocrine, Stockholm, Sweden). The mean eNO value (in ppb) of three technically acceptable attempts per flow rate was used for analysis. Alveolar eNO fraction (ppb) as well as the bronchial nitric oxide flux ($nL \cdot s^{-1}$) were calculated with a modification of the two-compartment model of nitric oxide exchange [22]. The test was performed on two occasions to train subjects in performing eNO tests correctly. The eNO values acquired during the third baseline visit (day 9) were used to analyse treatment effects.

Air trapping on expiratory computed tomography scan

Quantitative image analysis of computed tomography (CT) scans were performed at end-expiration (near residual volume (RV) level), both before and after bronchoprovocation, which reflects regional air trapping due to small airways obstruction [23, 24].

An inspiratory CT scan was acquired during a 10-s breathhold at full inspiration (near total lung capacity) at baseline. This was followed by a CT scan during a 10-s breathhold at end expiration, which approximates RV. Subsequently, an MCh

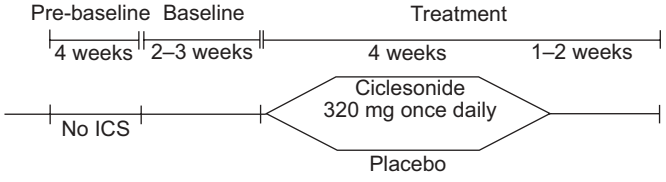

FIGURE 1. Schematic of the study design. During the baseline period, the procedures performed were as follows. Day 1: exhaled nitric oxide (eNO), single-breath nitrogen test (SBN_2) and methacholine (MCh) challenge; day 2: adenosine-5′-monophosphate challenge; day 9: eNO, SBN_2, MCh challenge and computed tomography (CT) scan; and day 16: bronchoscopy. During the final 1–2 weeks of the treatment period, the procedure performed were as follows. Day 44: eNO, SBN_2, MCh challenge and CT scan; day 45: AMP challenge; and day 52: bronchoscopy. ICS: inhaled corticosteroids.

provocation was performed on site, again followed by an end-expiratory scan immediately after PC_{20} of MCh had been reached. After 5 weeks of treatment, an end-expiratory scan after reaching PC_{20} of MCh was acquired again. Inspiratory and expiratory manoeuvres were practised twice before the procedure and subjects were coached by a trained technician during scanning in the supine position. All scans were performed on a 16-slice MultiDetector CT (MDCT) scanner (Siemens Somatom Sensation 16; Siemens AG Medical Solutions, Erlangen, Germany) at 120 kVp, 25 mAs (inspiration) and 30 mAs (expiration), 0.5 s rotation time. A table feed of 18 mm·rotation^{-1}, and a 1-mm slice thickness with 0.6-mm increment were used. The estimated effective radiation dose was 0.78 mSv for inspiratory scans and 0.94 mSv for expiratory scans.

Anonymised MDCT data were sent to the Center for Medical Diagnostic Systems and Visualisation (MeVis; Bremen, Germany) who was blinded to the intervention. Scan data were analysed by the advanced image analysis software MeVisPULMO3D. A detailed description of the lung segmentation with MeVisPULMO3D software is provided as supplementary data. Based on the segmentation, quantitative volumetric and densitometric analyses were performed of total, right and left lung separately and of each individual lung lobe. The parameters used in this study were volume (in mL), mean lung density (MLD; in Hounsfield units (HU)), 15th percentile density (in HU), and percentage of low attenuation areas (LAA). LAA were defined at a cut-off point of -950 HU. MCh-induced air trapping on CT was defined at baseline as the absolute change in MLD, 15th percentile density and LAA between the two expiratory scans before and after MCh challenge. The change in volume between the two expiratory scans before and after MCh challenge was also corrected for inspiratory lung volume by using the following equation:

$$\%\ \text{volume change} = 100 \times ((\text{inspiration-expiration}) - (\text{inspiration-expiration postMCh}))/(\text{inspiration-expiration}) \quad (1)$$

Closing volume with single-breath N_2 test

Closing volume measured with a single-breath nitrogen (SBN_2) test reflects air trapping due to small airways obstruction [25, 26]. At two baseline visits, 1 week apart, and after treatment, a SBN_2 test was performed (Quark PFT®; Cosmed, Rome, Italy). Subjects were coached into tidal breathing, after which they slowly inspired pure oxygen to total lung capacity. Hereafter, they slowly exhaled to RV level, during which the N_2 concentration was measured and plotted against lung volume. The slope of the alveolar N_2 plateau was calculated by one investigator by drawing the best-fit line through phase III of the expiratory volume–concentration curve. In order to minimise intra-observer variability, one reader measured closing volume (mL) and the slope of the alveolar N_2 plateau (δN_2 in %·mL^{-1}) on one day after all subjects had completed the study. Two measurements were selected for analysis when closing volume differed <20% or 100 mL. The mean of both measurements was used for analysis. The SBN_2 test was performed on two occasions to train subjects in performing the closing volume manoeuvre correctly. Closing volume and δN_2 values acquired during the second baseline visit (day 9) were used to analyse treatment effects.

ΔFVC% and ΔSVC% at PC_{20} of MCh and AMP

The percentage fall from baseline in FVC and slow inspiratory vital capacity (SVC) at the time 20% fall in FEV_1 occurred during bronchial hyperresponsiveness testing (ΔFVC% at PC_{20} and ΔSVC% at PC_{20}, respectively), may reflect air trapping due to excessive bronchoconstriction or small airways closure [27, 28].

MCh and AMP challenge testing was performed using the standardised 2-min tidal breathing protocol [29]. Additionally, FVC and SVC were measured in a combined manoeuvre (see online supplementary material) at 30 and 90 s after each inhaled dose of either MCh or AMP. Spirometry was measured with a daily calibrated dry wedge spirometer (Masterscope; Jaeger, Hoechberg, Germany). Subjects received doubling doses of MCh bromide (0.038–19.6 mg·mL^{-1}) at two baseline visits, 1 week apart, and after treatment (fig. 1). The subjects received doubling doses of AMP (0.04–320 mg·mL^{-1}) at baseline and after treatment. The fall in FVC and SVC (ΔFVC% and ΔSVC%) at PC_{20} was calculated using log-linear interpolation.

Cytokines measured in epithelial lining fluid in peripheral airways

The most direct method to assess airway inflammation is *via* bronchoscopy. The diameter of a bronchoscope is too large to reach the small airways but microsampling probes may reach the peripheral airways [30, 31]. The technique and cytokine measurements are described in the online supplementary material.

Statistical analysis

A Wilcoxon signed-rank test was used to assess within-treatment differences; a Mann–Whitney U-test was used for between-treatment differences (the difference between changes with ciclesonide and placebo treatment). All analyses were considered to be explorative in the absence of a statistical power calculation given the pilot nature of the study.

RESULTS

Study population

A total of 16 subjects were randomised to treatment and completed the study, seven subjects received placebo and nine received 320 μg of ciclesonide, once daily in the morning. Demographics and lung function at baseline of both groups were not significantly different (table 1).

Small airways parameters measured at baseline were also not statistically different between treatment groups (table 2), although higher values were observed in the ciclesonide group as a result of the randomisation of more males to this group.

Treatment effects

Alveolar eNO

Median (range) alveolar eNO values were significantly lower after ciclesonide (8.5 (3.7–12.5) ppb) than after placebo (16.5 (5.6–39.6) ppb; p=0.012). The median decrease in alveolar eNO from baseline with ciclesonide (4.4 ppb) was significantly different from the change from baseline with placebo (-0.4 ppb; p=0.006; fig. 2).

Air trapping on expiratory CT scan

MCh-induced air trapping at baseline is presented in table E1 in the online supplementary data.

TABLE 1 Patient characteristics and lung function at baseline and after treatment

	Placebo		Ciclesonide	
	Baseline	Post-treatment	Baseline	Post-treatment
Subjects n	7		9	
Male	2 (29)		5 (56)	
Age yrs	44 (21–53)		36 (19–56)	
BMI kg·m^{-2}	24 (19–30)		24 (20–28)	
FEV_1 % pred	97 (76–120)	91 (76–118)	88 (62–109)	98 (79–116)$^{\#,\P}$
FEV_1/FVC %	77 (68–88)	78 (66–83)	67 (52–79)	70 (60–83)
FVC % pred	101 (84–144)	105 (82–141)	115 (93–122)	117 (96–136)$^{\#,\P}$
SVC % pred	103 (82–145)	109 (81–145)	112 (94–131)	122 (98–135)$^{\#,\P}$
PC_{20} of MCh, mg·mL^{-1}	0.4 (0.2–4.2)	0.3 (0.1–3.6)	0.5 (0.1–2.0)	1.3 (0.2–39.2)$^{\#,\P}$
PC_{20} of AMP, mg·mL^{-1}	4.8 (0.2–23.1)	3.8 (0.7–23.4)	4.0 (0.2–36.2)	35.1 (1.2–640.0)$^{\#,\P}$
eNO at 50 mL·s^{-1} ppb	65 (34–204)	83 (28–222)	99 (33–281)	36 (17–59)$^{\#,\P}$

Data are presented as n (%) or median (range), unless otherwise stated. BMI: body mass index; FEV_1: forced expiratory volume in one second; % pred: % predicted; FVC: forced vital capacity; SVC: slow inspiratory vital capacity; PC_{20}: provocative dose causing a 20% fall in FEV_1; MCh: methacholine; AMP: adenosine-5′-monophosphate; eNO: exhaled nitric oxide. $^{\#}$: $p<0.05$ compared with placebo; ¶: $p<0.05$ compared with baseline.

Median (range) expiratory lung volume after MCh decreased by 59 (1,569– -117) mL with ciclesonide and increased by 121 (-20–236) mL with placebo, although these within-treatment differences were not statistically significantly. The changes in expiratory lung volume, MLD and 15th percentile density on expiratory CT scan after MCh challenge testing differed significantly between the ciclesonide and placebo group (between-treatment difference), $p=0.042$, $p=0.016$ and $p=0.023$, respectively (fig. 3a–c), whereas the change in percentage LAA was of borderline significance ($p=0.055$; fig. 3d).

Closing volume with SBN_2 test

Median closing volume decreased in both the placebo (140–105 mL) and ciclesonide group (230–115 mL). These decreases were not statistically significant, both within and between treatment groups (fig. 4).

ΔFVC% and ΔSVC% at PC_{20} of MCh and at PC_{20} of AMP

ΔFVC% and ΔSVC% at PC_{20} of MCh and at PC_{20} of AMP did not change significantly with either treatment.

$FEF_{25–75\%}$ % predicted

Mean forced expiratory flow between 25 and 75% of forced vital capacity ($FEF_{25–75\%}$) was measured with spirometry. Median $FEF_{25–75\%}$ % pred increased in the ciclesonide-treated group from 52–63%, which was of borderline significance ($p=0.051$). The change with ciclesonide was not significantly different from the change with placebo (table 2).

Secondary parameters

The mean increase in $\log_2$ PC_{20} of MCh was significantly larger with ciclesonide than with placebo, 1.4 *versus* 0.2 doubling doses, respectively ($p=0.031$). The mean increase in $\log_2$ PC_{20}

TABLE 2 Small airway parameters at baseline and after treatment

	Placebo		Ciclesonide	
	Baseline	Post-treatment	Baseline	Post-treatment
Subjects n	7		9	
Alveolar eNO ppb	14.7 (8.5–39.2)	16.5 (5.6–39.6)	17.3 (6.9–67.3)	8.5 (3.7–12.5)$^{\#,\P}$
$FEF_{25–75\%}$ % pred	63 (34–87)	61 (54–86)	52 (29–66)	63 (30–97)$^{+}$
Closing volume SBN_2 mL	140 (95–495)	105 (60–430)	230 (60–820)	115 (35–975)
ΔFVC % at PC_{20} of MCh	13.6 (4.9–15.3)	13.2 (2.5–19.4)	12.4 (6.1–16.8)	12.7 (5.6–19.7)
ΔFVC % at PC_{20} of AMP	12.2 (5.4–14.3)	14.1 (9.0–18.9)	12.0 (3.5–17.2)	12.3 (4.1–15.9)
Total expiratory lung volume on CT after MCh mL	2993 (2158–4636)	2973 (2368–4916)	4165 (2262–5576)	3831 (2338–5166)$^{\#}$

Data are presented as median (range), unless otherwise stated. eNO: exhaled nitric oxide; $FEF_{25–75\%}$: mean forced expiratory flow between 25 and 75% of forced vital capacity; SBN_2: single-breath nitrogen test; ΔFVC: change in forced vital capacity; PC_{20}: provocative dose causing a 20% fall in forced expiratory volume in one second; MCh: methacholine; AMP: adenosine-5′-monophosphate; CT: computed tomography. $^{\#}$: $p<0.05$ compared with placebo; ¶: $p<0.05$ compared with baseline; $^{+}$: $p=0.051$ compared with baseline.

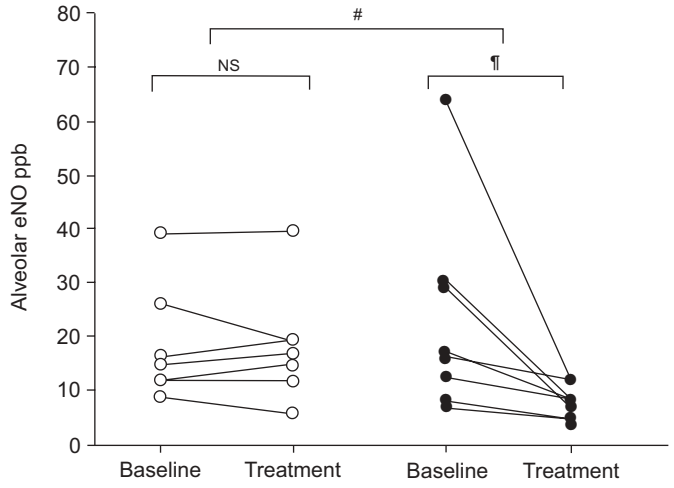

FIGURE 2. Alveolar exhaled nitric oxide (eNO). Alveolar eNO before and after treatment with placebo (○) and ciclesonide (●). NS: nonsignificant. #: p=0.006 from Mann–Whitney U-test; ¶: p=0.012 from Wilcoxon signed rank test.

of AMP was also significantly larger with ciclesonide than placebo, 2.9 *versus* 0.3 doubling doses, respectively (p=0.029). The changes in FEV_1 % pred, FVC % pred and SVC % pred were also significantly larger with ciclesonide (median increase of 6, 6 and 4% pred, respectively) than with placebo (median decrease of 2, 2 and 1% pred, and p=0.003, p=0.003 and p=0.023, respectively). Bronchial median (range) eNO decreased significantly with ciclesonide (1.4 (0.2–5.6) $nL \cdot s^{-1}$; p=0.016), significantly more than with placebo (p=0.004).

Cytokines measured in epithelial lining fluid in peripheral airways

Bronchoscopy was performed in seven subjects (two placebo and five ciclesonide). Due to blood contamination in 21 (50%) out of a total of 42 peripherally placed probes, cytokine measurements of peripherally sampled epithelial lining fluid (ELF) were only possible in one subject in the placebo and two in the ciclesonide group, both at baseline and post-treatment. Thymus and activation-regulated chemokine was not detectable in any of the probes. Other cytokine concentrations in ELF are presented in table E2 of the online supplementary data. Statistical analysis was not performed due to the small sample size.

DISCUSSION

The present pilot study demonstrates that treatment with 320 μg ciclesonide once daily can specifically improve parameters reflecting inflammation and patency of the small airways, even in a small sample of 16 patients with mild-to-moderate asthma. Earlier studies have already shown the efficacy of ciclesonide in maintaining asthma control and in reducing symptoms and airway hyperresponsiveness [9–13, 16, 17, 32–36]. The present study confirms and extends these observations in that ciclesonide also exerts anti-inflammatory effects on small airways. The present study has demonstrated the beneficial effects of the small-particle ICS, ciclesonide, on small airway involvement in asthma compared with placebo. Further studies are needed to evaluate whether treatment with this small-particle ICS is superior to treatment with a large-particle ICS, with respect to small airway involvement, symptoms and control of asthma. If so, the importance of ICS distribution throughout the whole lung must be acknowledged when treating patients who do not gain optimal asthma control with conventional large-particle ICS.

The present study is the first to assess the effects of ciclesonide on small airway parameters. VERBANCK *et al.* [37] demonstrated beneficial effects of small-particle ICS on acinar lung zone abnormalities in asthma but did not compare these effects with placebo. HAUBER *et al.* [38] previously demonstrated beneficial effects of a small-particle ICS on peripheral airways by a reduction of eosinophilic inflammation in transbronchial biopsies and an increase in $FEF_{25–75\%}$ % pred. In a study from the same group, signs of airway remodelling were reduced, thus demonstrating direct effects of a small-particle ICS on peripheral airway inflammation, remodelling and airway function [39]. Nevertheless, transbronchial biopsies are not easily applicable in clinical practice due to their invasive nature. In a less invasive manner, large- and small-particle ICS were investigated for effects on small airways by assessing MCh-induced air trapping on expiratory HRCT scans in mild-to-moderate asthma. The small-particle ICS improved MCh-induced air trapping significantly more than the large-particle ICS, indicating a direct effect of the former on the small airways [40]. Consistent with these results, ZEIDLER *et al.* [23] demonstrated that montelukast (leukotriene receptor antagonist) reduced MCh-induced air trapping on expiratory CT scans in mild-to-moderate asthma, in association with improved quality of life. However, other parameters of small airway dysfunction, such as closing volume (SBN_2 test), were not related to the montelukast-induced reduction of air trapping [23]. The present study confirms that treatment with a small-particle ICS significantly reduces MCh-induced air trapping due to small airway closure. Additionally, the present authors found the effects of the small-particle ICS ciclesonide on an even less invasive marker of small airway inflammation, *i.e.* alveolar eNO. This is an interesting finding since alveolar eNO is an easy to measure, noninvasive parameter that is preferable to CT scanning or transbronchial biopsies for clinical follow-up of small airway inflammation.

A possible limitation of the present CT methodology may be the lack of spirometric gating. Therefore, theoretically, one can never be entirely sure that end-expiratory lung volume is not affected by things such as an incomplete expiration, and thus the lack of spirometric gating may affect reproducibility of the measurements. Nevertheless, other studies that also do not apply spirometric gating have demonstrated that air trapping on HRCT is significantly associated with spirometric indices of global and peripheral airway obstruction [24]. This indicates that even without spirometric gating, CT scanning is still very sensitive in assessing small airways disease in asthma. Although spirometric gating is of value when measuring air trapping on a baseline expiratory scan, the use of spirometric gating in assessing MCh-induced air trapping in asthma could be questioned. Due to MCh-induced air trapping, lung inflation may occur and residual volume may increase, thereby affecting the trigger to scan. The subjects in the present study were trained in order to perform maximal inspiration and maximal expiration correctly and the inspiratory/expiratory manoeuvres were closely observed during scanning. Scans were only acquired when inspiratory/expiratory manoeuvres were technically satisfactory.

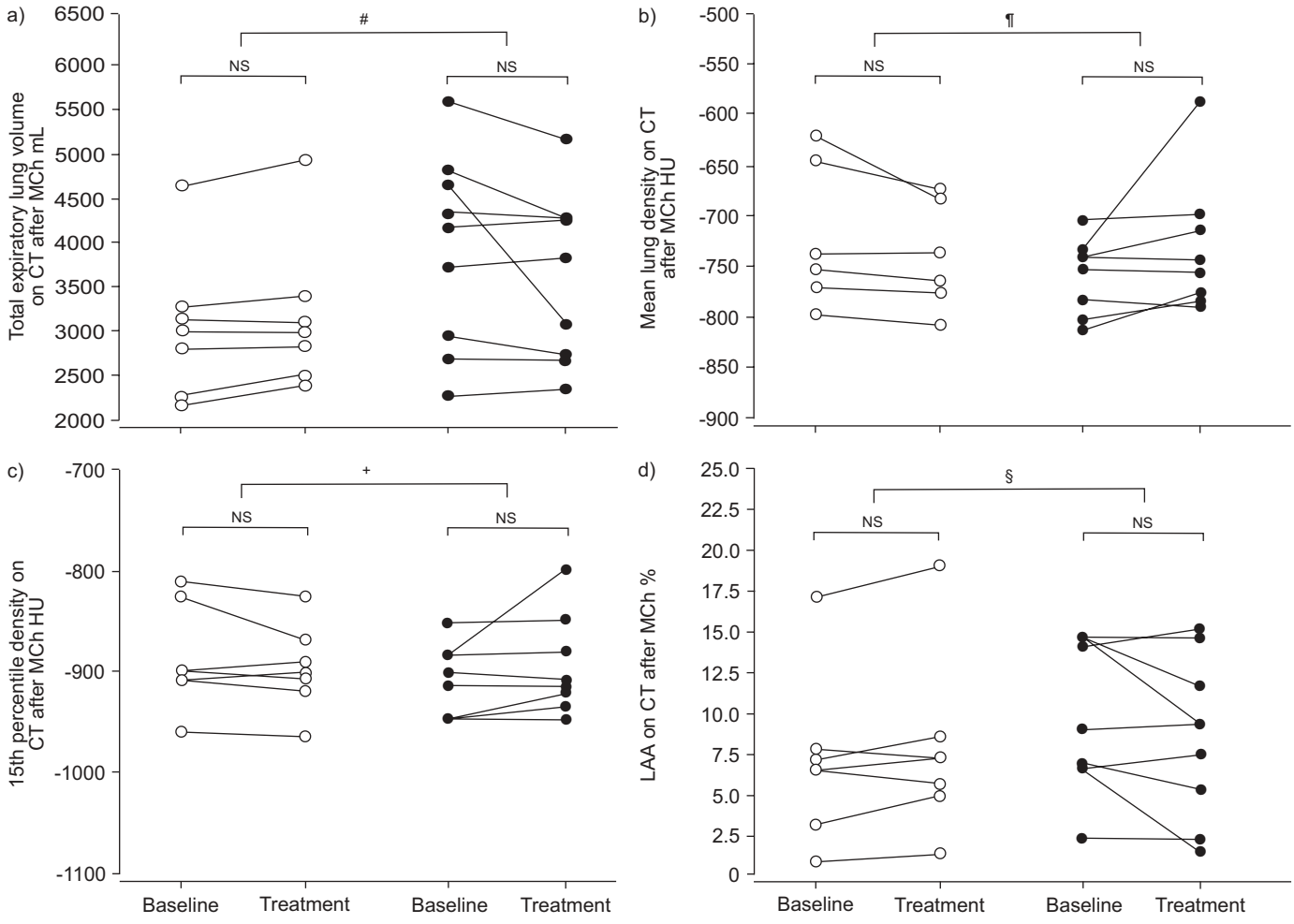

FIGURE 3. Methacholine (MCh)-induced air trapping on computed tomography (CT) scans. MCh-induced air trapping on CT before and after treatment with placebo (○) and ciclesonide (●). a) Total expiratory lung volume after MCh; b) mean lung density after MCh; c) 15th percentile density after MCh; and d) percentage low attenuation areas (LAA) after MCh. #: p=0.042; ¶: p=0.016; +: p=0.023; §: p=0.055. All p-values from Mann–Whitney U-test.

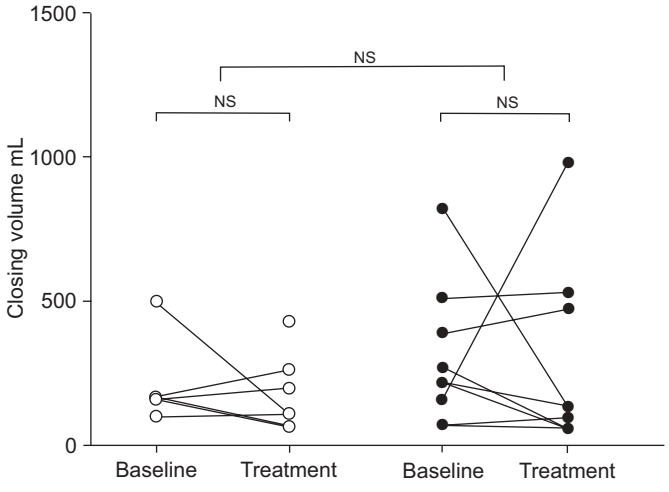

FIGURE 4. Closing volume with the single-breath nitrogen test after treatment with placebo (○) and ciclesonide (●). NS: nonsignificant.

One of the pitfalls of small airway research in asthma is the absence of a gold standard to compare small airway function and or inflammation to. Nevertheless, all tests used in the present study have been extensively investigated and suggested by other research groups as adequate parameters to reflect functioning of the small airways, which justifies the choice of these tests [21, 25, 28, 29, 41]. Furthermore, the finding in the present study that a small-particle ICS improves small airway parameters in a small number of asthmatics, in contrast with placebo, provides a sound basis for the validity of these parameters.

Why would ciclesonide significantly improve alveolar eNO and MCh-induced air trapping on expiratory CT but not the other small airway parameters evaluated? First, some of the small airway parameters tested had a large variability (table 2), thus reducing statistical power. A formal power calculation was not performed in this pilot study as effect sizes of small airway parameters were unknown when the study was designed. Nevertheless, it is reassuring that other intervention studies

providing positive effects of small-particle ICS have used similar sample sizes [38, 42, 43]. Secondly, the lack of improvement in closing volume after ciclesonide treatment does not rule out a beneficial effect on small airway closure, as SVC improved in the present patients; therefore, closing capacity may have been a better measure than closing volume. However, this could not be examined due to the lack of lung volume measurements. Another explanation for the results may be that the tested small airway parameters do not all measure the same aspects of small airway disease. KING and co-workers [44, 45] described that closing volumes measured using the SBN_2 test can differ greatly between two individuals who have a similar extent of airway closure and air trapping on a CT scan, because the SBN_2 test detects airway closure during expiration at a lung volume that is different from the end-expiratory lung volume. VAN VEEN *et al.* [21] described that ΔFVC at PC_{20} of MCh was not associated with alveolar eNO in contrast to other measures of peripheral airway dysfunction. The present authors conclude that future studies are needed in order to determine which is the best tool to monitor small airway improvements; for now, eNO and expiratory CT scanning are promising.

Seven subjects underwent a bronchoscopy at baseline and after treatment during which ELF from central and peripheral airways was sampled with microsampling probes. Half of all sampled probes were contaminated with blood from bronchial mucosa. The present authors conclude that it is not feasible to sample ELF from peripheral airways in clinical studies investigating asthma patients, in contrast to successful application in acute respiratory distress syndrome and COPD [31, 32].

In summary, the present study has demonstrated that treatment with the small-particle inhaled corticosteroid ciclesonide, at a once daily dose of 320 μg, improves alveolar exhaled nitric oxide and methacholine-induced air trapping on expiratory computed tomography scan in patients with mild-to-moderate asthma. The present findings suggest that alveolar exhaled nitric oxide is a useful tool to assist in diagnosing and monitoring small airway pathology in asthma, since it is already sensitive to changes in a small number of patients. It has already been demonstrated that ciclesonide reaches the small airways [8], and the present study provides evidence, for the first time, that ciclesonide exerts anti-inflammatory effects at this site.

ACKNOWLEDGEMENTS

The authors would like to thank M. Farenhorst, J. Bruins-Slot, K. Vink-Klooster, M. Swierenga and S. Alberts-Poots from the Lung Function Laboratory for performing all lung function tests, W. Tukker from the Dept of Radiology for acquiring the computed tomography scans, A. Smidt from the Dept of Endoscopy for assistance during bronchoscopy, B. Dijkhuizen and A. van Oosterhout from the Laboratory of Allergology and Pulmonary Diseases for analysing the microsampling probes (all University of Groningen, Groningen, the Netherlands), and C. Hilck, S. Zentis, V. Dicken and J-M. Kuhnigk from MeVis Diagnostic Systems (Bremen, Germany) for post-processing computed tomography scans

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